Project Report: Genome–Genome Integration: Symbiosis, genetic assimilation, and evolutionary innovation

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Project Progress

Our broader goal is to elucidate changes in gene content and expression patterns that catalyze the establishment and diversification of genome–genome interactions. Using insect–associated bacteria as model systems, we are examining the molecular andevolutionary forces that shape endosymbiotic associations. Our studies target Proteobacterial species that represent both long–term, stable mutualisms and transient parasitic interactions. This research extends our previous project, "Microbial symbionts: Agents for reorganizing genome architectures," under NASA grant NCC2–1054.

Obligate mutualists are ideal models to study extremes of bacterial evolution, as they represent the most radical retooling of genome size and structure known in the prokaryotic world, and include the smallest known prokaryotic genomes (as small as 450 kb, and typically < 1 Mb). Yet, their close phylogenetic relationship to well–characterized free–living bacteria such as *Escherichia coli* enables direct comparisons across species with distinct lifestyles. Our results to date elucidate how genome interactions affect molecular evolution and genome architecture in these long–term, stable mutualisms (Fry and Wernegreen, submitted). We published two population genetic studies showing exceptionally strong effects of mutational pressure and genetic drift in obligate endosymbionts (Herbeck et al. 2003, Wernegreen and Funk, in press). In addition, we completed a collaborative, multivariate analysis of codon and amino acid usage of the five published mutualist genomes (Schaber et al. submitted). This study extends our *Wigglesworthia* genome analysis published last year, and shows variable evolutionary rates and amino acid usage among functional categories.

In the past year, we successfully completed and closed the full genome sequence of *Blochmannia* associated with *Camponotus pennsylvanicus* (i.e., *B. pennsylvanicus)* , totaling 19,480 reads (15.4 Mb total) f rom a short–insert genomic library to obtain 12–fold coverage of this 792–kb chromosome. With consultation of Dr. Monica Riley's group at the Marine Biological Laboratory (MBL), we are using extensive genetic, biochemical and metabolic information available for *E. coli* to refine automatic annotation and metabolic pathway

predictions in this and other endosymbiont genomes. Comparison with the published *B. floridanus* genome shows several differences in gene content; however the two genomes otherwise show complete synteny indicating exceptional genome stability throughout the ~20 MY since they diverged. Building on this genome data, we have identified several candidate "key symbiotic genes" that may mediate bacteria–eukaryotic associations. We have successfully applied reverse transcriptase, quantitative PCR to measure the relative expression of certain loci, including biosynthetic and housekeeping loci of *Blochmannia* .

Standard methods of phylogenetic reconstruction are based on models that assume homogeneity of nucleotide composition among taxa. However, this assumption is often violated in actual datasets. We are examining possible effects of nucleotide heterogeneity among lineages on phylogenetic reconstruction, and aim to develop computationally feasible and rigorous approaches to account for variable %GC content in systematics (Herbeck et al. submitted) . Our initial focus has been intracellular and free–living species in the gamma–Proteobacteria, but the approaches developed should prove useful for studying any group with variable modes and patterns of sequence evolution.

Our research on *Wolbachia* explores genome dynamics in this reproductive parasite that represents a dynamic genome–genome interaction. Our synthetic review article of the recently–published Wolbachia genome notes its distinctly higher plasticity compared to stable mutualist genomes (Wernegreen, 2004). By understanding mechanisms of this plasticity, we hope to elucidate processes that drive this endosymbiont's exceptionally wide host range and diverse biological roles. Using molecular phylogenetic approaches, we have documented lateral transfer and recombination of an active bacteriophage among Wolbachia strains, and developed a novel hypothesis on the exchange of genetic information between intracellular microbial communities (Bordenstein and Wernegreen, in press). Using quantitative PCR, we have successfully measured the relative densities of phage, Wolbachia, and host genomes across several insects showing different degrees of Wolbachia–induced reproductive alterations. These quantitative studies suggest a direct role of phage in symbiont phenotypes. These and other *Wolbachia* projects largely extend upon the NAI/NRC Fellowship of Dr. Seth Bordenstein, and are described in more detail in his separate report.

Highlights

- Genome comparisons within clades of stable mutualists show complete synteny, indicating a lack of inversions, transpositions, or gene acquisition by lateral transfer. This striking genome stability contrasts sharply with labile genomes of free–living or parasitic bacteria.
- Although AT mutational bias and genetic drift affect all loci in endosymbiont genomes, the distinct effects of natural selection at functionally significant genes offers a valuable computational tool to identify pathways that are critical to the bacteria and/or its host.
- Bacteriophages of Wolbachia play a significant role in the genome flux of this endosymbiont. These mobile elements are widespread among Wolbachia, transfer laterally between divergent Wolbachia strains, and

may shuttle chromosomal genes. Preliminary quantitative analysis of phage and Wolbachia dynamics suggests that phage may play role in Wolbachia−induced alterations of insect reproduction.
- Due to extreme heterogeneity in base composition among free−living and endosymbiotic bacteria, the use of nonhomogenous models of sequence evolution that acount for variable GC content provides significant improvement in estimating their phylogentic relationships.

Roadmap Objectives

- ***Objective No. 4.2:*** Foundations of complex life
- ***Objective No. 5.1:*** Environment−dependent, molecular evolution in microorganisms
- ***Objective No. 5.2:*** Co−evolution of microbial communities
- ***Objective No. 6.2:*** Adaptation and evolution of life beyond Earth